# Compensatory Hypertrophy after Unilateral Ovariectomy and Destruction of Follicles in the Anestrous Ewe[1] (36051)

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Mallampati and Casida (1) failed to find compensatory development of follicles after unilateral ovariectomy in the anestrous ewe. The failure was explained in part on the possibility that anestrous ovaries secrete only small amounts of estrogen. Removal of one ovary therefore removes very little inhibition and compensatory change does not occur in the remaining ovary. Growth of new ovarian follicles in ewes during the breeding season was demonstrated within 4 days after destruction of all macroscopic follicles visible on the surface of the ovary (2, 3). The initial hypothesis in the present study was that destruction of all macroscopically visible follicles in anestrous ewes might lower the estrogen level enough to result in growth of new follicles (Expt. 1). In testing that hypothesis, however, growth of new follicles was found to occur after destruction of follicles on only one ovary which seemed inconsistent with the inability of Mallampati and Casida (1) to demonstrate compensatory hypertrophy after unilateral ovariectomy. It was then hypothesized that destruction of follicles in one ovary affects growth of follicles in the untreated ovary differently than unilateral ovariectomy (Expt. 2).

*Materials and Methods. Expt. 1.* On May 28 and 29, 1970, twenty mature white-faced ewes were assigned randomly to four treatment groups: (i) sham-operated; (ii) follicles destroyed in the right ovary; (iii) follicles destroyed in the left ovary; (iv) follicles destroyed in both ovaries. For independent comparisons of different treatment groups, three ewes within each group were assigned randomly for studying the left ovary and the other two for studying the right ovary. It was thus a $2 \times 2 \times 2$ factorial experiment, the factors being: (i) experimental ovary: follicles intact or follicles destroyed; (ii) studied ovary: follicles intact or follicles destroyed; (iii) side: left or right ovary. The total number of follicles $\geq$ 1 mm and the diameter of the largest follicle were recorded at autopsy. Transformation of these data was made to the $\log_{10} \times + 1$ and to the $\log_{10}$. respectively, for statistical analysis.

*Expt. 2.* On June 4 and 5, 1970, eighteen mature Hampshire ewes were assigned randomly to six treatment groups: (a) sham operated where three sheep were used for studying the left ovary; (b) sham operated where three other sheep were used for studying the right ovary; (c) follicles destroyed in the left ovary; (d) follicles destroyed in the right ovary; (e) left ovary removed; (f) right ovary removed. The total number of follicles $\geq$1 mm, and the diameter of the largest follicles were recorded at autopsy. When the untreated ovary of sheep that had the other ovary removed or its follicles destroyed was studied, comparisons were made for the total number of follicles $\geq$1 mm (transformed to $\log_{10}$), diameter of the largest follicle, and number of follicles 6–8 mm. However, when the studied ovary had been treated (follicles destroyed) comparisons were made only for the total number of follicles $\geq$1 mm and the diameter of the largest follicle.

In the two experiments, general anesthesia was used and the ovaries and uterus were

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TABLE I. Effect of Destruction of Follicles in Anestrous Ewes on Follicular Development (Expt. 1).[a]

| Treatment[b] | Side of treatment | Ovary studied | No. of ewes | Follicular fluid wt (mg) | No. of follicles ≧1 mm | Diam of largest follicle (mm) |
|---|---|---|---|---|---|---|
| Follicles destroyed in one ovary | Right | Right | 2 | 194 | 2.5 | 2.0 |
| | | Left | 3 | 368 | 13.6 | 4.3 |
| | Left | Right | 2 | 419 | 13.5 | 5.5 |
| | | Left | 3 | 236 | 6.6 | 2.0 |
| Follicles destroyed in | Right + left | Right | 2 | 163 | 2.0 | 2.0 |
| | | Left | 3 | 381 | 9.3 | 4.3 |
| Controls | | Right | 2 | 295 | 11.0 | 4.0 |
| | | Left | 3 | 349 | 9.3 | 6.0 |

[a] All macroscopically visible follicles were cauterized, and ewes were autopsied 4 days later.
[b] Mean squares are given in Table II.

exposed via a midventral laparotomy. None of the ewes was found to have CL at surgery, confirming the anestrous condition. Destruction of follicles was performed as previously described (2). All ewes were autopsied 4 days after surgery and the ovaries were removed for study. Data recorded for each ovary were the diameter of each grossly visible follicle ≧1 mm, and total follicular fluid weight (FFW). FFW was estimated for each ovary by taking the difference between the intact ovarian weight and that of the ovarian tissue after mincing with a razor blade and blotting with a paper towel.[3]

*Results. Expt. 1.* Table I gives the mean data of follicular development obtained in the studied ovaries, whether untreated or treated and whether left or right ovaries. Growth of new follicles occurred in the ovaries that had their follicles destroyed. However, the number of follicles ≧1 mm and diameter of the largest follicle in the treated ovaries was not as great as in the untreated ovaries (treated ovary vs untreated ovary, $p<.01$, Table II).

Growth of new follicles in the treated ovaries did not differ statistically (Table II) whether the follicles were destroyed in one or two ovaries (one treated ovary vs two treated ovaries as measured in the treated ovaries).

[3] Total ovarian weight prior to mincing varied from 494 to 1927 mg and the FFW from 103 to 704 mg.

Slightly greater follicular development was seen in the untreated ovary of sheep that had one ovary treated compared to those that had both ovaries serving as controls. However, none of the measures of increased follicular development were significant (control ovaries vs one treated ovary as measured in the untreated ovary, Table II). The sum of one treated and one untreated ovary from sheep which had follicles destroyed in the ovary was 608 mg FFW; whereas the sum of both ovaries from untreated sheep was 644 mg. Compensatory changes had reestablished approximately the control level of follicular development for the whole animal.

*Expt. 2.* All sheep presented in Table III are also presented in Table IV. In Table III, the treated ovary is studied; in Table IV, the ovary opposite the treated ovary is studied.

Growth of new follicles occurred in the treated ovary that had its surface follicles destroyed. FFW, number of follicles ≧1 mm, and diameter of the largest follicle (Table III) were significantly less, however, in the ovary that had its follicles destroyed than in control ovaries.

Table IV gives the values obtained in the contralateral ovary of sheep that had one ovary removed or follicles destroyed in one ovary. The FFW per ovary in the untreated-studied ovary averaged over the unilateral-ovariectomy and the follicles-destroyed groups

TABLE II. Analysis of Variance of Ovarian Measurements of Anestrous Ewes with Destroyed Follicles (Expt. 1).

| Source of variation | DF | Mean squares: Follicular fluid weight $\log_{10}$ | Diam of largest follicle $\log_{10}$ | No. of follicles $\geq$1 mm $\log_{10} X+1$ |
|---|---|---|---|---|
| Ewes with one treated ovary vs those with two treated ovaries as measured in treated ovaries | 1 | .008 | .060 | .009 |
| Ewes with one treated ovary vs those with two treated ovaries $X$ side as measured in treated ovaries | 1 | .126 | .060 | .255 |
| Ewes with one treated ovary vs those with control ovaries as measured in untreated ovaries | 1 | .025 | .000 | .040 |
| Ewes with one treated ovary vs those with control ovaries $X$ side as measured in untreated ovaries | 1 | .004 | .061 | .010 |
| All treated ovaries vs all untreated ovaries | 1 | .143 | .401[a] | .775[a] |
| All right ovaries vs all left ovaries | 1 | .045 | .036 | .088 |
| All treated vs all untreated ovaries on the right compared to all treated vs all untreated on the left side | 1 | .085 | .025 | .175 |
| Error | 12 | .031 | .035 | .078 |

[a] $p < .01$.

was greater ($p<.05$) than for the comparable ovaries of the control groups. Significant differences for the FFW in the untreated-studied ovary were not found between unilateral ovariectomy and unilateral-destruction of the follicles. The diameter of the largest follicle was not changed significantly by the treatment. However, the number of 6–8 mm follicles was increased significantly, compared to the number in comparable control ovaries.

The total FFW in the animal averaged 729 mg for the control ewes, 531 mg for the unilateral ovariectomized ewes, and 706 mg for the ewes with follicles destroyed in one ovary.

*Discussion.* As observed in Expt. 1, growth of new follicles occurred after destruction of all macroscopically visible follicles in one or two ovaries. Since the amount of growth in a treated ovary was similar whether follicles had been destroyed in one or both ovaries, it is assumed that the same amount of gonadotropins was released for the two types of treatments.

Expt. 2 was designed to test the hypothesis

TABLE III. Effect of Unilateral Destruction of Follicles on Growth of New Follicles (Expt. 2).[a]

| Treatment | Ovary studied | No. of ewes | Follicular fluid wt (mg)[b] | No. of follicles $\geq$1 mm[b] | Diam of largest follicle[b] |
|---|---|---|---|---|---|
| Follicles destroyed on left ovary | Left | 3 | 225 | 1.67 | 1.0 |
| Control | Left | 3 | 344 | 9.33 | 6.7 |
| Follicles destroyed on right ovary | Right | 3 | 284 | 3.67 | 2.3 |
| Control | Right | 3 | 385 | 10.67 | 6.7 |

[a] All macroscopically visible follicles present on one ovary were cauterized and ewes were autopsied 4 days later.

[b] Effects: Control vs ovary with follicles destroyed, for FFW, number of follicles $\geq$1 mm and diameter of largest follicle: $p < .05$, $p < .001$, and $p < .025$, respectively. Error mean square: FFW, 9828; number of follicles, 15.83; diameter largest follicle, 0.75.

TABLE IV. Effect of Unilateral Ovariectomy and Unilateral Destruction of Follicles During Anestrus in Ewes on Follicular Development (Expt. 2).[a]

| Treatment | Ovary studied | No. of ewes | Follicular fluid wt (mg)[b] | No. of follicles ≧1 mm | Diam of largest follicle | No. of follicles 6–8 mm |
|---|---|---|---|---|---|---|
| Left ovary removed | Right | 3 | 554 | 11.67 | 6.67 | 2.00 |
| Follicles destroyed on left ovary | Right | 3 | 475 | 9.67 | 7.00 | 2.33 |
| Control | Right | 3 | 385 | 9.33 | 6.67 | 1.33 |
| Right ovary removed | Left | 3 | 509 | 10.00 | 7.67 | 2.67 |
| Follicles destroyed on right ovary | Left | 3 | 429 | 13.33 | 6.33 | 1.33 |
| Control | Left | 3 | 344 | 10.67 | 6.67 | 0.67 |

[a] Unilateral ovariectomy, or cauterization of all macroscopically visible follicles present on one ovary was done on 4th and 5th of June and ewes were autopsied 4 days later.

[b] Effects: follicular fluid weight: control vs (ovariectomy + destruction of follicles)/2, $p < .05$; error mean square, 9828. Number of follicles 6–8 mm: control vs (ovariectomy + destruction of follicles)/2, $p < .05$; error mean square, 0.94.

that destruction of follicles on one ovary affects growth of follicles in the untreated ovary differently than unilateral ovariectomy affects growth of follicles in the remaining ovary. No difference was found between the effect of unilateral ovariectomy on the retained ovary and the effect of unilateral destruction of follicles on the untreated ovary. However, when the total FFW in the animal was measured and comparison was made between control ewes, unilateral-ovariectomized ewes, and ewes with follicles destroyed in one ovary, the original FFW was less nearly approached in the animals from which one ovary had been removed. Brinkley and Young (4) compared follicular development following unilateral ovariectomy and unilateral destruction of follicles in cycling pigs. The total number of follicles and the FFW per ovary or per animal was the same whether one ovary had been removed or follicles had been destroyed previously.

The significant increase in FFW observed in the intact ovary after unilateral ovariectomy or destruction of follicles appeared to be due to greater number of 6–8 mm follicles in treated animals than in control animals.

It is recognized that the estimate of FFW includes relatively small amounts of other ovarian fluids freed by the mincing procedure such as lymph. Importantly, however, they also include fluid from small vesicular follicles which cannot be enumerated or measured readily.

The present experiments demonstrated compensatory hypertrophy resulting from unilateral ovariectomy contrary to Mallampati and Casida (1) report. Their experiments were done at approximately the same time of the year. The experiments differed in that the autopsy was done 10 days after surgery in their study, whereas in the present study the autopsy was done 4 days after surgery. There was also a difference in breeds.

Estrogen was presumably produced by follicles of anestrous ewes, since halving the number of follicles led to compensatory changes. The main pituitary gonadotropin released after destruction of follicles would appear to be follicle-stimulating hormone (FSH), since growth of new follicles in the treated ovary and stimulation of follicles in the untreated ovary were observed, but no new ovulation points were observed in any case.

*Summary.* Compensatory ovarian changes were studied in anestrous ewes by unilateral ovariectomy or destruction of follicles in one ovary by cauterization. Growth of new follicles occurred within 4 days in ovaries that had their follicles destroyed but the resulting

follicular development was not as great as in untreated ovaries ($p < .01$). The sum of ovarian follicular fluid weight (FFW) of one treated (follicles cauterized) and one untreated ovary was approximately as great as the sum of both ovaries of control ewes. The FFW in the untreated ovary of ewes with follicles destroyed in the opposite ovary and the FFW of the remaining ovary of unilaterally ovariectomized ewes was greater ($p < .05$) than the FFW of comparable ovaries of control groups. No significant differences were found between the remaining ovary in unilateral ovariectomized ewes and the untreated ovary in ewes with follicles destroyed in one ovary.

It is concluded that ovarian compensatory changes take place in the anestrous ewes, which are consistent with the hypothesis that increased release of gonadotropins resulted due to decreased levels of estrogens following hemicastration or destruction of follicles.

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